Appendix to Chapter 4: Details of Laboratory and Analytical Uses of ODS

A1 Laboratory uses of ODS

The laboratory and analytical uses of methyl bromide and other ODS were discussed in the 2006 Progress Report of the TEAP. The 2008 TEAP Progress report contained details of a number of these procedures and the non-ODS alternatives, and the 2009 TEAP progress report contained an extensive tabulation of methods using ODS and approved or recommended non-ODS alternatives.

Whether the ODS is recovered, consigned for destruction (together with other laboratory wastes) or released to the environment, the quantities involved are trivial when compared to those involved in chemical industry, although there is no accounting of them by Parties.

A1.1 Laboratory preparative uses

(i) methyl bromide Methyl bromide has often been the reagent of choice for reactions in which the methyl group, CH_3-, is transferred from bromine to an atom such as oxygen, nitrogen, phosphorus or magnesium. The most common case is that of formation of quaternary ammonium salts:

$$R_3N \quad + \quad CH_3\text{-}Br \quad \rightarrow \quad R_3N\text{-}CH_3^+ \quad + \quad Br^-$$

Here, R represents other groups attached to the nitrogen. Ether linkages are established by reaction with oxygen, for example CH_3-O-R, but in the case of phosphorus the initial salt is reacted further (by reaction with base, to remove HBr) to an ylid containing the $=CH_2$ function:

$$R_3P \quad + \quad CH_3\text{-}Br \quad \rightarrow \quad R_3P\text{-}CH_3^+ \quad + \quad Br^- \quad \rightarrow \quad R_3P{=}CH_2$$

The ylid may be reacted further to transfer the $=CH_2$ moiety to another molecule. Sulfur-based ylids are also known: they are prepared and used in ways analogous to the phosphorus ylids.

Methyl bromide reacts with magnesium to form methyl magnesium bromide, CH_3-Mg-Br, from which the methyl group may be transferred to another molecule, and a new carbon-carbon bond established.

For all of these reactions there are alternatives to the use of methyl bromide, such as methyl chloride (chloromethane), methyl iodide, trimethyl phosphate and various methyl sulfonate esters. Cost and availability are not barriers to uptake of the alternatives, but long-term users of methyl bromide in these applications may need to experiment so as to adapt their practice to the alternative methylating agents.

A critical use exemption for ‘preparative’ uses of methyl bromide, in which the ODS is destroyed, was included in decision XVII/10.

(ii) carbon tetrachloride (CTC) As with methyl bromide, these uses involve transfer of all or part of the CTC molecule to another molecule. A common reaction is the addition of CTC, via a free radical mechanism (involving and initiator radical and radical fragments $Cl^\cdot$ And $Cl_3C^\cdot$), to a carbon-carbon double bond:

$$CCl_4 \quad + \quad \text{-CH=CH-} \quad \rightarrow \quad Cl_3C\text{–CH-CH-Cl}$$

Except that a more complicated alternative synthesis pathway be adopted, possibly more complex and expensive, there are no alternatives to the use of CTC in such reactions.

In the Appel reaction, CTC reacts with triphenyl phosphine in a complicated way to replace the –OH group in an alcohol with chlorine while the CTC is converted to chloroform (trichloromethane):

$$PPh_3 + CCl_4 + RCH_2OH \rightarrow OPPh_3 + RCH_2Cl + CHCl_3$$

There is an analogous reaction with carbon tetrabromide, with bromine replacing chlorine in the above reaction scheme.

(iii) difluoromethylation with HCFC-22 HCFC-22, $CHClF_2$, may be used to transfer a –CF_2 group to another molecule, for example, an alkoxide formed from an alcohol:

$$RO^- + CHClF_2 \rightarrow RO\text{-}CHF_2 + Cl^-$$

This reaction can also be performed with halon 22B1, $CHBrF_2$, by displacement of bromine.

(iv) reactions with bromochloromethane (BCM) Reactions in which a halogen such as chlorine or bromine is displaced from the molecule of a controlled substance, with formation of a new bond between the carbon atom and a carbon, sulfur, oxygen or nitrogen atom, can be performed with BCM. It is even possible to perform stepwise reactions, with the bromine being displaced first, and the chlorine next (under more vigorous conditions):

$$R(1) + CH_2BrCl \rightarrow R(1)\text{-}CH_2\text{-}Cl$$

$$R(2) + R(1)\text{-}CH_2\text{-}Cl \rightarrow R(1)\text{-}CH_2\text{-}R(2)$$

There is an example in the industrial production of the antibiotic sultamicillin, for which the use BCM is used in large excess in the first step.

A1.2 Laboratory solvent uses

(i) CTC in chemical reactions CTC has been widely used as a solvent in synthetic organic chemistry for reactions in which two or more components are dissolved in the solvent and heated to form new substances. These are recovered by cooling followed by appropriate 'work up' often involving evaporation (and potential recovery) of the CTC. CTC is used in the chemical industry for one or more of the following reasons: reasonably good solvency, does not attack common materials including many elastomers used in reaction vessels, non-flammable and not easily degraded under conditions of use, easily removed by evaporation or distillation without excessive energy consumption, readily available at affordable prices. Many of the industrial uses of CTC stem from patented procedures that were developed in laboratories. Where such laboratory work is destined to become an industrial process, consideration needs to be given to finding an alternative solvent at the outset. Trichloroethylene, which is not a controlled substance, meets many of the above criteria but must be handled with care because of toxicity concerns that also apply to CTC.

The one chemical reaction that the CTOC has been able to discover in which no alternative has been found for CTC is its use as a solvent in bromination reactions using N-bromosuccinimide (depicted below as succ-N-Br):

$$Succ\text{-}N\text{-}Br + CH_3\text{-}C{=}C\text{-} \rightarrow Succ\text{-}N\text{-}H + BrCH_2\text{-}C{=}C\text{-}$$

The reaction, conducted under mild conditions (with boiling CTC), is specific for conversion of –CH groups to –CBr when the CH is in an allylic position (one carbon away from a double bond or aromatic ring) as shown. During the reaction, the soluble Succ-N-Br is replaced by insoluble Succ-N-H as the reaction proceeds, and it is thought that this differential solubility in CTC is important to the success of the reaction. Some experiments have been conducted to identify a suitable alternative solvent, but so far without success.

(ii) chain transfer during polymerization Small quantities of CTC are included in reaction mixtures for free-radical-induced polymerization of methyl methacrylate to polymethyl methacrylate. The CTC functions as a chain-transfer reagent, stopping the growth of polymer chains with concomitant initiation of a new polymer chain. The overall effect is to limit chain size and so influence the properties of the resulting polymer.

Other chain transfer agents are available, and newer methods of polymerization are available that obviate the need for this technology. However, conservatism in the laboratory is likely to see only slow movement away from the use of CTC.

(iii) CTC in spectroscopy The use of CTC in analytical spectroscopy is detailed in section 3.2.1 below. However, there are non-analytical uses of this type that find a place in teaching and research laboratories. When an infrared spectrum of the C-H stretching region, say 2800-3200 cm^{-1}, is required, CTC has commonly been used as a solvent for the substance under investigation. Alternatives are tetrachloroethylene, as outlined below, fluorocarbons such as S-316, or for specialist applications, carbon disulfide (although the low boiling point, 46^{o}, and unpleasant odour of carbon disulfide act to limit its use).

For recording nuclear magnetic resonance (NMR) spectra arising from hydrogen (1H) nuclei in the sample under investigation, CTC has been used a solvent since its molecules have no hydrogen atoms and consequently do not contribute a background spectrum. However, CTC does not have sufficient solvent power to dissolve many substances, and so the use of deuteron-chloroform – $CDCl_3$, chloroform in which the hydrogen has been replaced by deuterium – has become more frequent although it is somewhat more expensive. Given the widespread availability and use of deuteron-chloroform, and its counterparts such as deuteron-acetone, the use of CTC in this application is easily replaced by alternatives.

CTC is also used as solvent for the recording of Raman spectra, a technique in which the sample is irradiated with ultraviolet light, and comparison is made between the light emerging and that of the incident beam. Differences in the two are due to characteristic vibrational spectrum of the sample being investigated, and in order to visualize the spectrum, a solvent that has no Raman spectrum is required. CTC meets this need, since its molecules are symmetrical and have no dipole moment, thus rendering its Raman spectrum as zero. It is often the case that infrared and Raman spectra are recorded for the same sample, and where C-H vibrations give rise to the bands of interest the solvent employed must not interfere. Carbon disulfide, and deuterated solvents are recommended as alternatives to CTC (B. Schrader, ed., *Infrared and Raman Spectroscopy. Methods and Applications*, VCH, Weinheim, 1995, pages 146-7 and 220-222).

(iv) grease removal and washing of NMR tubes Removing grease from glassware, especially the smooth surfaces of standard taper joints, may be done with paper or fabric wetted with a solvent, and CTC is one solvent that has been used in this way. Numerous alternatives are available, depending on the type of grease encountered, paraffin or synthetic, for example. Readily available solvents such as hexane or other petroleum hydrocarbons, chloroform and aromatic solvents such as benzene may be used at similar cost and under the same occupational health and safety conditions.

The sample tubes used in nuclear magnetic resonance (NMR) experiments are long (15 cm) and narrow (5 or 10 mm diameter), and they need to be washed to remove the last sample before they reused. In the case of ^{1}H NMR there is an advantage in using CTC because any traces of the washing solvent remaining in the tube will not affect the subsequent use, since CTC has no hydrogen atoms in its molecule. When ^{13}C spectra are being recorded, all traces of carbon-containing solvents – and that includes all organic solvents – must be removed. The best method involves rinsing the tubes with acetone, and then drying them (upside down to allow the escape of heavy vapors) in a ventilated oven at temperatures near 100°C.

(v) iodine partition and equilibrium experiments One of the traditional experiments in undergraduate physical chemistry laboratories concerns the distribution of iodine between an organic solvent, immiscible with water, and water or a solution containing other species that can react with iodine, and CTC was commonly prescribed as the organic solvent in this experiment. Two sets of measurements are made: (i) concentration of iodine in solvent and water after the two have been shaken to enable equilibrium distribution of the iodine; (ii) concentration of iodine in the solvent and of iodine and the complex ion I_3^- in the water phase. The calculation from the measured data gives the distribution coefficient for iodine in the solvent/water pair, and the equilibrium constant for the reaction

$$I_2 + I^- \leftrightarrows I_3^-$$

While older laboratory manuals prescribe the use of CTC as the organic solvent for this experiment, subsequent writers (for example, www.practicalchemistry.org/experiments) have used cyclohexane, the only significant difference being that, in the latter case, the solvent is less dense than water and forms the upper layer, whereas CTC is more dense and forms the lower layer. Clearly, other solvents such as chloroform or trichloroethylene (both denser than water) or hexane (less dense) could be used in this experiment.

A2 Analytical Uses of ODS

A2.1 ODS used as a solvent for spectroscopic measurements

(i) recording infrared and nuclear magnetic resonance spectra
The most common uses in this category are of CTC or CFC-113 which are used as solvents when measurements are being made by nuclear magnetic resonance or infrared spectroscopy. In the spectroscopic regions of interest, the solvent makes no contribution and so the contributions of the solute molecules are clearly visible and their intensities can be directly related to the concentrations of the solutes. The analyses may concentrate on the presence in the solute (sometimes called the analyte) of certain atoms or bonds that are not present in the solvent, and may be used to measure quantities of single substances, of mixtures, or of certain functional groups in molecules, as is the case with the hydroxyl index (OH groups measured).

Two specific analyses are discussed in (ii) and (iii) below, but this general exclusion may apply to other analytical methods, not all of which would be acknowledged standard methods, so analysts should be aware that alternatives to the use of ODS can be found in all cases.

(ii) hydrocarbons (oil and grease) in water or soil or oil mists in air
The molecules of these solvents have no carbon-hydrogen bonds, and so the infrared spectra are clear in the region where such bonds absorb, approximately 2900-3300 cm^{-1}. The molecules of oils and greases are rich in C-H bonds and vibrations of these bonds give rise to absorption peaks in this region of the infrared spectrum. The intensities of these absorption peaks are directly related to the concentration of oil or grease in the solution, and can be prepared with the intensities of peaks from solutions prepared with known concentrations that are used to calibrate this instrumental analysis.

The standard methods of analysis based on the use of CTC or CFC-113 as solvent are elegant in their simplicity. The sample is prepared for analysis by shaking the contaminate water with the solvent, or (in the case of soils and absorbent materials on which oil mists have been deposited) extracting with the solvent. The resulting solution of oil or grease in CTC or CFC-113 is dried and an infrared spectrum is recorded. Peak intensities are compared with standards. CTC is used as solvent in method ASTM D-3921 (total hydrocarbons extracted from water, wastewater and sediments), and in method APHA AWWA-WPCF 5520C (IR method) for hydrocarbon extraction from water and soils. Despite the fact that CFC-113 was already a controlled substance, ASTM method D3921-96, proposing its use, was introduced in 1996, and revised in 2003 (ASTM D3921-96(2003)e1).

The analysis may also be performed with tetrachloroethylene (perchloroethylene) as solvent, because this substance meets the necessary criteria of molecular structure and physical properties (solvent power and water-immiscibility) and is available in spectroscopic grades of high purity. Industrial grades of this solvent have been widely used in the dry cleaning industry. Health and safety aspects are about the same as those with CTC, and both of these chlorinated solvents need to be used with appropriate laboratory care. Although there is no standard method that describes the use of tetrachloroethylene, its use has been reported in the scientific literature and it has been introduced successfully as a replacement for CTC in the petroleum refining industry of Chile. Literature details may be found in: Farmaki, E., Kaloudis, T., Dimitrou, K., Thanasoulias, N., Kousouris, L and Tzoumerkas, F.,'Validation of an FT-IR method for the Determination of Oils & Greases in Water, with the use of tetrachloroethylene as the extraction Solvent', *Proceedings of the 9th International Conference on Environmental Science and Technology*, Rhodes Island, Greece, 1-3 September 2005. Farmaki, E., Kaloudis, T., Dimitrou, K., Thanasoulias, N., Kousouris, L and Tzoumerkas, F., 'Validation of an FT-IR method for the Determination of Oils & Greases in Water, with the use of tetrachloroethylene as the extraction Solvent', *Desalination*, 2007, 210 (1-3), 52-60.

The proprietary solvent S-316 (the dimer/trimer of chlorotrifluoroethylene) produced by Horiba has also been used successfully in a number of laboratories for the spectroscopic analysis of oil and grease. It is more expensive than other solvents but has very low toxicity and may be preferred on those grounds. A new standard method ASTM D 7066-04 (*Test Method for Dimer/Trimer of Chlorotrifluoroethylene S-316 Recoverable Oil and Grease and Nonpolar Material by Infrared Determination*) (Rintoul, S. (2005)) describes the use of this solvent.

There are several alternative, non-spectroscopic methods for analysis of oils and greases in these contexts. The most rudimentary, for which standard analysis exist, is a gravimetric method (*Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 20th edition, Washington, DC. Method 5520C (Partition-infrared. Method 5520B gravimetric method. US EPA (1978). US EPA (1978), *Method 418.1: Petroleum Hydrocarbons, Total Recoverable*, Storet No. 45501. ASTM (2003). ASRM (2003), *Method D 3921-96: Standard Test Method for Oil and Grease and Petroleum Hydrocarbons in Water*, ASTM International.) which involves the extraction of the oil or grease into a volatile hydrocarbon solvent (most often hexane), evaporation of the solvent and weighing of the residual non-volatile oily material. Volatile hydrocarbon fractions may be lost during this procedure, whereas they would be included in the spectroscopic analysis described above. However, the gravimetric method is cheaper – not having to rely on a spectroscopic instrument – and in the case of environmental samples there volatile material is likely to have been lost before the sample is collected for analysis, so the gravimetric method gives a true account of the quantity being analysed.

The most sophisticated method available for these analysis similarly collects the oil into an organic solvent and then analyses the mixture by GC-MS, a combination of gas chromatography (in which hydrocarbon components of the mixture are separated and quantified) and mass spectroscopy (in which their molecular nature is revealed). The USEPA Method 8260B 'Volatile organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)' is a suitable method.

The use of ODS for 'testing of oil, grease and total petroleum hydrocarbons in water' was eliminated from the global exemption by Decision XI/15 (clause (a)) from 2002, but such uses have persisted, especially in developing countries. The availability of alternatives to ODS was further recognized in the TEAP Progress report 2009 and in Decision XXI/6.

(iii) simethicone (polydimethylsiloxane)
Polydimethylsiloxane is used as an internal medicine, marketed as Dimethicone (or Dimeticone) or, when mixed with silica, as Simethicone or Simeticone. The active ingredient can be identified and its concentration may be measured by infrared spectroscopic analysis. However, instead of monitoring peaks due to absorption by C-H vibrations, as in the case of the oil and hydrocarbon analyses discussed above, the analysis of the polydimethylsiloxane uses the intensity of the peak for vibration of the CH_3-Si group near 1260 $cm^{-}1$. Various references give the peak position 1254, 1259 and 1260 and 1261 cm^{-1}, and 7.9 µm = 1266 cm^{-1}, but the spectrometer resolution is usually on the order of 4 cm-1 so these figures are essentially the same. The solvent originally prescribed for this analysis in a number of standard reference works and in a research paper (G. Torrado, A. Garcia-Arieta, F. de los Rio, J.C. Menéndez and S. Torrado, 'Quantitative determination of dimethicone in commercial tablets and capsules by Fourier transform infrared spectroscopy and antifoaming activity test', *J. Pharmaceutical and Biomedical Analysis*, 1999, **19**, 285-292) was CTC, which has no absorption peaks close to 1260 cm^{-1}. Many other solvents in which the polydimethylsiloxane would be soluble would also have no absorptions near the peak to be measured, and both toluene and chloroform have been used in this analysis. Both the US Pharmacopeia 2009 (Vol 2, page 3555) and the British Pharmacopoeia 2010 (Vol 2, pages 1892-3), which also incorporates the requirements of the European Pharmacopoeia 6th edition, describe the use of toluene for the assay, and the latter reference also includes the spectrum of Dimeticone (Vol 4, page S41).

The availability of alternatives to ODS in this analysis was reported in the 2009 TEAP Progress report and recognized in Decision XXI/6.

A2.2 ODS used as a solvent for electrochemical methods of analysis

(i) cyanocobalamin For the assay of cyanocobalamin, the British Pharmacopoeia 2010 (pages 607-608) recommends ultraviolet and visible absorption spectrophotometry of an aqueous solution (peak at 361 nm); thin layer chromatography using a mixed solvent (dilute ammonia, methanol and dichloromethane) after application of an aqueous ethanol solution of cyanocobalamin; and liquid chromatography using as mobile phase a mixture of methanol and aqueous disodium hydrogen phosphate. Similar conditions for liquid chromatography of radio-labelled cyanocobalamin[^{57}Co] and [^{58}Co] are given on pages 3383-3384.

The same conditions for liquid chromatography of radio-labelled cyanocobalamin are prescribed the US Pharmacopeia 2009 (pages 2008-2009) but the cobalamin radiotracer assay described on page 145 employs mixtures of cresol-carbon tetrachloride and butanol-carbon tetrachloride, followed by elution from an alumina column and measurement of the absorbance at 361 nm. In view of the later information contained in the British Pharmacopoeia, the use of organic solvents could be avoided.

(ii) bromine index In a procedure used to measure the degree of unsaturation of hydrocarbon fractions by determining the uptake of bromine, the chemical reaction being represented by the equation:

$$-CH=CH- \quad + \quad Br_2 \quad \rightarrow \quad -CHBr\text{-}CHBr-$$

The bromine is generated electrolytically *in situ* from bromide and bromate ions and its disappearance is monitored electronically, in a mixed solvent containing methanol and acetic acid (the components that hold inorganic salts in solution and promote conductivity) and 1,1,1-trichloroethane (the component that holds the hydrocarbon in solution). There are several standard methods for this assay, including ASTM D2710-99 'Standard Test Method for Bromine Index of Petroleum Hydrocarbons by Electrometric Titration' and ASTM Method D1159 'Test Method for Bromine Number of Petroleum Distillates and Commercial Aliphatic Olefins by Electrometric Titration', which describes the solvent as a mixture of glacial acetic acid, methanol, sulfuric acid and 1,1,1-trichloroethane. The procedure for determination of bromine Index closely resembles that used for Karl Fischer determination of traces of water, and the same apparatus may be used for both determinations, an example being the 796 Titroprocessor marketed by Metrohm.

The need to replace1,1,1-trichloroethane, an ozone depleting substance, had already been identified by the companies that market the analytical equipment for this specialised measurement. Methylene chloride, diethyl carbonate and 1-methyl-2-pyrrolidone were suggested as suitable alternatives. One bulletin (Metrohm method 9) describes a mixture of glacial acetic acid, 1-methylpyrrolidone, methanol and sulfuric acid. A bulletin from another branch of the company (Metrohm, Application Bulletin 177/4e) describes the use of 1,1,1-trichloromethane but also comments that 'if possible one should refrain from using chlorinated solvents. Our investigations have shown that carbon tetrachloride and 1,1,1,-trichloroethane can be replaced by diethyl carbonate'. Further information may be found in Metrohm Application Bulletin 177/4e. Automatic determination of the bromine index and/or bromine number in petroleum products. (www.metrohm.co.uk/bulletins/177_e.pdf, accessed January 2008.

Metrohm. Method 9 – Bromine index of heptane.
www.metrohm.com/products/01/pac/oilpac/e/oilpac_method9_e.pdf, accessed January 2008.

The petroleum refining industry in Chile tested the alternatives and concluded that methylene chloride was the best alternative to 1,1,1-trichloromethane.

A2.3 Analyses involving selective solubility in the ODS

(i) cascarosides A number of medicines consist of, or are prepared from, natural material, and analytical procedures have been developed for measuring the amount of the active constituents in the natural materials. Cascarosides are bright yellow substances found in the bark of the tree *Rhamnus pushiana* DC (cascara bark, cascara sagrada) and they possess laxative properties. Cascarosides are listed in many pharmacopeias and the content of active constituents is prescribed (for example, European Pharmacopeia (Vol. 2), Council of Europe, Maisonneuve S.A., Sainte-Ruffine, France, 1971. p. 355, and USP 25 I NF 20 - The United States Pharmacopeia; The National Formulary. 2002. Rockville, MD: The United States Pharmacopeial Convention, Inc.).

A common assay involves extraction of the active constituents with an organic solvent, followed by thin layer chromatography. The yellow spots on the chromatogram are compared for size with those of a standard solution of the active constituents. A solvent commonly used for this procedure is CTC but many other solvents can be used instead. For example, alcohols such as ethanol or iso-propanol, and solvents such as dichloromethane or ethyl acetate. A more advanced chromatographic method for this analysis has also been described (De Witte, P., Cuveele, J. and Lemli, J. 'Determination of bicascarosides in cascara fluid by high-performance liquid chromatography', *Journal of Liquid Chromatography*, 1991, **14**, 2201-2206)

There is also an instrumental method in which the intensity of absorption of visible light in a spectrophotometer, by a solution of the active constituents in ethyl acetate, is used. So, in summary, many common organic solvents can replace CTC and a number of analytical methods are available.

(ii) thyroid extracts A further example of the use of CTC in extraction of active constituents from natural materials is an application to thyroid glands removed from animals. This is an assay for the thyroid hormones which is based on the work of L.W. Riggs one hundred years ago ('The Determination of Iodine in Protein Combinations', *Journal of the American Chemical Society*, 1910, **32**(5), 692-698).

Extraction of particular components from natural materials is not confined to medicinal products but also plays a part in research projects, and this is covered in the 'laboratory' section, above.

(iii) polymers An ODS, typically CTC, could have found application as a solvent for selectively removing a polymer from a mixture, so that proportion of that component in the mixture could be measured, or so that the infrared of ^{1}H nuclear magnetic resonance (NMR) spectrum of the polymer could be recorded for assay or identification purposes. However, very few polymers are soluble in such solvents and, in any case, other solvents can be found for these purposes. For recording NMR spectra, solutions of polymers in trifluoroacetic acid or hot *o*-dichlorobenzene have been used. Another kind of solvent use would be to remove some soluble impurity or additive (a plasticizer or flame retardant, for example) from a commercial polymer, for identification or assay purposes, but many other solvents are available for such purposes.

(iv) formation of picrates Older methods for the identification of unknown organic substances are still practiced in some teaching laboratories in developing countries, whereas in developed countries and in the chemical industry these methods have been displaced by modern spectroscopic methods. These are more expensive and hence the survival of the old methods in developing countries. The identification of the unknown substance can involve its reaction with another chemical substance to form a 'derivative'. The melting point of this derivative can be used, by referenced to standard tables and in conjunction with other properties of the unknown substance, to identify the unknown substance.

Under this procedure, polycyclic aromatic hydrocarbons such as naphthalene, anthracene, phenanthrene and other aromatic hydrocarbons can be reacted with picric acid to form 1:1 adducts known as picrates. Picric acid and the unknown substance are dissolved in a suitable solvent by warming, and upon cooling the picrate is recovered as crystals. CTC has often been used as the solvent, but most textbooks (for example, F.J. Smith and E. Jones, *A Scheme of Qualitative Organic* Analysis (1951); W.J. Hickinbotham, *Reactions of Organic Compounds* (1942); A.I. Vogel, *A Textbook of Practical Organic Chemistry* (several editions) prescribe the use of benzene, acetone, ethanol or acetic acid and so alternatives to the use of CTC are readily available, cheap and safe to use. Similar procedures can be adopted for the formation of analogous derivates of hydrocarbons with picrolonic acid or 1,3,5-trinitrobenzene.

A2.4 ODS used to preconcentrate the analyte

A further development of the solvent extraction procedure described above is the dissolution, often involving pre-concentration, of a soluble or volatile substance prior to its introduction into the analytical instrument. Four examples are considered below.

(i) liquid chromatography (HPLC) of drugs and pesticides The concentrated solution of the substance or mixture (the analyte = material to be analyzed) to be analyzed, is dissolved in a suitable solvent and injected into the chromatography column. The flow of solvent to develop the chromatogaphy – commonly a methanol-water or an acetonitrile-water mixture – is then passed through the column, eluting the components of the analyte and separating them. They may be identified in or recovered from the emerging solution. While CTC has been used in this application, many other solvents can replace it and CTC is not listed as a suitable solvent in the major texts and laboratory manuals (W.J. Lough and I.W. Wainer, eds., *High Performance Liquid Chromatography. Fundamental principles and Practice*, Blackie Academic & Professional, London, 1996, pages 168-169; M.C, McMaster, *HPLC. A Practical User's Guide*, VCH, New York, 1994, page 137; S. Kromidas, ed., *HPLC Made to Measure. A Practical Handbook for Optimization*, Wiley-VCH, 2006, pages 350-351). According to Lough and Wainer, the sample should be 'dissolved in the mobile phase', or if this is not feasible, 'in a liquid that is chemically very similar to the mobile phase', and this makes CTC an unlikely choice. For pre-c0ncentration of the sample, polar solvents such as dichloromethane or the Folsch mixture (2:1 chloroform:methanol) are recommended.

(ii) gas chromatography of organic chemicals such as steroids Similar techniques are used in this analysis, the difference being that the chromatogram is developed, and components thus separated, by a stream of inert gas (nitrogen or argon). The separated components may be trapped as they are eluted from the column. Again, many solvents are available for use in transferring the analyte to the chromatography column.

(iii) adsorption chromatography of organic chemicals Once again the aim of the analyst is to transfer the analyte to the chromatography column in the minimum volume of solvent and then to develop the chromatogram by successive elution with solvents. As in the two examples above, a range of suitable solvents is available for transferring the analyte to the column.

(iv) atomic absorption spectroscopy The aim of this analysis is to identify metals by subjecting them to intense heat in a flame or plasma and examining the light emitted at particular frequencies, characteristic of each element. The light intensity can also be used to make a quantitative analysis (= assay). Metal salts are typically soluble in water (or dilute acid) and so can be introduced into the hot zone in the form of aqueous solutions. A technique for pre-concentration of dilute samples, or when trace quantities are involved, is to make a solvent-soluble complex of the metal ion, dissolve the complex in a suitable organic solvent, and begin the analysis with this. While CTC has been used in this application, together with the complexing agents dithizone, a dithiocarbamate or 8-hydroxyquinoline, it is more common to use chloroform, methyl isobutyl ketone, xylene, or a phosphate ester (G.F. Kirkbright and M. Sargent, *Atomic Absorption and Fluorescence Spectroscopy*, Academic press, London, 1974, pp 49-497. *Preconcentration Techniques for Trace Elements*, CRC Press, 1992, p 124).

(v) X-ray fluorescence Sample preparation is the same for a related technique for metal analysis, in which the sample is irradiated with high energy X-rays or gamma rays, and the emitted (secondary) X-rays are recorded and analyzed for the presence of frequencies characteristic of the various metals.

A2.5 Titration of iodine with thiosulfate (iodometric analyses)

Volumetric analysis or titration, in which a reagent of known concentration is added from a burette, to a sample to be analyzed, has been the backbone of laboratory analysis for well over a century and still finds a place in many laboratories despite the availability and sophistication of modern instrumental methods of analysis. Of course the old technique is inexpensive, except for the time of the analyst, and that is one reason why it has persisted. The most common applications of the technique involve acid-base reactions, the acid normally in the burette and the base, together with an indicator substance that will change colour at the equivalence point ('end point' of the titration), in the receiving flask. Electrometric methods can also be used to detect the end point.

In the titrations under consideration here, the reagent in the burette is sodium thiosulfate, $Na_2S_2O_3$, and the solution in the flask contains iodine I_2, the quantity of which is to be measured in the volumetric analysis. Because the iodine has limited solubility in water, it is normally held in solution as the more soluble I_3^- ion, which releases iodine as the titration progresses. During the titration the iodine is converted (reduced) to iodide ion, I^-, and the brown colour of the iodine fades gradually to yellow and disappears at the end point.

The final change from pale yellow to colourless can be hard to detect by eye and so two methods have been developed for visualizing it. In the first of these, as the end point approaches and iodine concentration is already low, a solution of starch is added so that a deep blue starch-iodine complex forms in solution. When the last traces of iodine are gone, the blue colour disappears. The second visualization method is based on the intense violet colour of iodine solutions in organic solvents that have no oxygen in their molecules. CTC has been used in this method. A few mL are added to the titration solution in the flask, forming a globule at the bottom (since CTC is much denser than water) and dissolving the iodine. As the iodine is removed from the solution during the titration, the violet colour fades and the end point – colourless – is easily detected.

In this second visualization method, CTC (relative density 1.59) can be replaced with another solvent that has no oxygen and also meets the requirements of water immiscibility and density. Chloroform ($CHCl_3$, relative density 1.48), dichloromethane (CH_2Cl_2, 1.33) and trichloroethylene (C_2HCl_3, 1.46) all meet these criteria, and chloroform has been found to be quite successful. Like CTC, these solvents can be recovered and reused, and this is often done if a laboratory has many such titrations to perform. The use of chloroform as an alternative to CTC is described in standard texts such as Vogel's *Quantitative Inorganic Analysis*.

There are many applications that rely on the generation of iodine in solution, in equivalent to the amount of the substance being analyzed. Some representative examples are given below.

(i) iodine Titrations of the kind described above are performed in a number of situations where iodine is handled. One example is the monitoring of iodine content in medicinal preparations containing iodine as disinfectant or antiseptic. Another is the assay of residual iodine in water from which solid iodine has been removed in the commercial production of iodine from mineral deposits. Chloroform, has been shown to serve well in this application.

(ii) copper In the volumetric method for determination of copper in solution, the copper is reacted with iodide ion, oxidizing it so that a quantity of iodine, equivalent to the amount of copper, is generated in the solution. The copper is concomitantly reduced, changing its oxidation state from Cu(II) to Cu(I). The quantity of iodine is determined by means of the titration described above and thus the quantity of copper can be calculated. As before, the iodine may be titrated with sodium thiosulfate to the end point that can be detected with starch or an organic solvent such as chloroform. There is no necessity for the use of CTC since alternatives are available.

(iii) arsenic Arsenic, in its pentavalent As(V) state – that is, the form of arsenate ion, $As_3O_4^{3-}$, or arsenic pentoxide, As_2O_5 - can be determined in more or less the same way, since its reaction with iodide ion converts an equivalent amount of iodide to iodine, the arsenic being reduced to the trivalent (+3) state. As before, the iodine may be titrated with sodium thiosulfate to the end point that can be detected with starch or an organic solvent such as chloroform. There is no necessity for the use of CTC since alternatives are available.

(iv) hypochlorite, chlorate and bromate Each of these species is a powerful oxidizing agent, capable of converting iodide ion to iodine in amount equivalent to the quantity of oxidizing agent. The quantity of iodine, and therefore of the oxidizing agent, can be determined as above by titration with thiosulfate.

(v) sulfur Sulfur can be determined by means of a related volumetric method that involves converting the sulfur to sulfur dioxide or sulfite ion, SO_3^{2-}, and titration with iodine, which oxidizes the sulfite to sulfate, SO_4^{2-}. The procedure is the converse of the above titrations, and is properly termed iodimetry (a term frequently misused in the literature). The end point, the first appearance of unconsumed iodine in solution, may be detected by the methods described above for monitoring the disappearance of iodine in the process of iodometry – that is, blue colour with starch, or violet colour in an organic solvent. An excellent example is provided in a recent article (M. Gros, J-P. Morand and A. Bezos, 'Determination of Total Sulfur Contents in the International Rock reference Material SY-2 and other Mafic and Ultramafic Rocks Using an Improved Scheme of Combustion/Iodometric Titration', *Geostandards and Geoanalytical Research*, 2007, **29**(1), 123-130) where starch indicator is used. Determinations of this type are sometimes performed by 'back titration' with thiosulfate following the addition of a known excess of iodine to the sulfur-containing solution, but the approximate amount of sulfur must be known in order for the excess to be calculated. Sulfur may also be determined by instrumental methods such as X-ray fluorescence (XRF), pyrohydrolysis/ion chromatography, inductively coupled plasma mass spectrometry (ICP-MS), and electron microprobe analysis (EMPA).

A2.6 Iodine Index (iodine value, iodine number)

The Iodine Index is an indicator of the extent to which the molecules being studied possess –C=C- linkages (double bonds or unsaturation). Such unsaturation may be in hydrocarbons, but the petroleum industry generally uses the closely related Bromine Index, rather than the Iodine Index, to express the degree of unsaturation in their products. The Iodine Index is commonly used when the unsaturation is in the components of natural fats and oils, and so edible oils and fatty foods such as butter are studied in this way, and products such as biodiesel that are derived from fats and oils.

The methodology involves the addition of a solution of iodine chloride (ICl) in acetic acid – named after the inventor of the technique as 'Wijs Solution' – to a solution of a weighed amount of the oil or fat in an organic solvent. Since a wide range of sample may need to be analysed, with differing solubilities, the mixed solvent ensures that the whole sample is in solution. CTC was for many years the organic solvent used in the Wijs method but a number of other solvents can be used as alternatives.

An excess of Wijs solution is used in the determination. The rate of the chemical reaction - addition of the ICl to the double bonds - is slow, and so a waiting time of at least an hour is necessary. After this time, an aqueous solution of potassium iodine is added and this reacts with the excess ICl to form iodine. The amount of iodine is measured in the usual way, by titration with sodium thiosulfate and visualization of the end point (when all of the iodine has been consumed by the thiosulfate) by starch, which forms a deep blue colour with iodine. The end point is the disappearance of the blue colour. Since the amount of Wijs reagent was known, and the excess was measured (as iodine) the quantity of ICl consumed can be calculated. The amount of sample is known, so the Iodine Index may be calculated.

The Association of Official Analytical Chemists has published a standard procure for determination of the Iodine Value using CTC (AOCS method Cd 1-25, (1989) but even at the time the method was being developed by the American Oil Chemists' Society (AOCS) it was acknowledged that CTC would have to be replaced and (later) that CFC-113 was not recommended as a replacement 'because of environmental concerns'. Subsequently, AOCS

Recommended Practice Cd 1b-87 gave cyclohexane as the best replacement for CTC but acknowledged that 'erratic results may be obtained for oils with iodine values' in the range 100-120.

ASTM D5768-02 (2006) is another standard methods for performing this analysis. The ASTM 'method was developed in order to replace the hazardous solvent, carbon tetrachloride, used in Test Method D 1959 with the less hazardous and more available solvents, iso-octane and cyclohexane. As data on the satisfactory use of other solvents becomes available, this test method will be amended to include those solvents'. The German organization Deutsches Institut fur Normung E.V. has developed method DIN 53241-1: 1995 Determination of iodine value - Part 1: Methods using Wijs solution, but details are not available.

A recent publication describes a method in which the unsaturated material being tested is reacted with iodine in a water-alcohol mixture at 50°C, with excess iodine being measured by thiosulfate titration as in the standard method. The method revives a procedure first described in 1924, and compares the results favourably with those achieved with the standard method described above. The new method is not (yet) a standard method although it was published in the AIST journal and so has been brought to the attention of the standard-setting organization (J.A. Aricetti, A.J. da S. maciel, O.C. Lopes and M. Tubino, 'A Simple Green method for Biodiesel Iodine Number Determination', *Journal of ASTM International* **7** (1), 1-8 (2010).

A2.7 Miscellaneous analyses

(i) stiffness of leather Earlier indications that ODS were used in this standard method were in error and so this procedure (ASTM D2821) is not of concern.

(ii) jellification point CTC is used in small quantities in the measurement of the jellification point of the seaweed-derived product, agar. As far as can be ascertained, this use is confined to a single company, where CTC is used because it is heavy (relative density 1.59) and immiscible with water. Following discussion with a CTOC co-chair, the company is seeking and should be able to find an alternative (non-ODS) that meets its requirements.

(iii) cement analysis This analysis was described in the 2008 TEAP Progress Report, in which it was observed that the original standard method using CTC had been replaced by another (ASTM C 243-95 Standard test method for Bleeding of Cement Pastes and Mortars) using 1,1,1-trichloroethane (TCA), but since this was also a controlled substance the method was withdrawn in 2001. There has been available for many years the standard test ASTM C 188-44, revised in 1967, in benzene is used as solvent and CTOC has received information that this method is acceptable to the industry. Hence, there is no justification for the continued use of CTC or TCA.

(iv) gas mask cartridge breakthrough As noted in the 2009 TEAP Progress Report, Japanese researchers have found that cyclohexane is a suitable alternative to CTC in the standard test of breakthrough time of a gas mask cartridge (M. Furuse, S. Kanno, T. Takano and Y. Matsu, 'Cyclohexane as an Alternative Vapor to Carbon Tetrachloride for the Assessment of Gas Removing Capacities of Gas masks', *Industrial Health*, **39**, 1-7 (2001). 'Carbon tetrachloride (CCl_4) vapor has long been used', the authors observe, ' as a representative organic vapor for testing breakthrough times of gas mask cartridges and canisters in the National Approval Test of Respirators as well as in respirator tests in manufactures'. However, a suitable alternative, cyclohexane, is available.

(v) porosity of activated carbon Impurities in gases or liquids can be removed by adsorption onto the surface of 'activated carbon', a kind of char that is produced by heating woody material in the absence of oxygen. Some of the best grades are made from coconut husks. The activated carbon has very high porosity, and therefore very high surface area, and this important property can be measured by measuring the uptake (g/100 g) of an organic chemical substance by grains of the activated carbon. The ASTM Method of long standing, D3467-04 (2009), Standard Test Method for Carbon Tetrachloride Activity of Activated Carbon, describes how this can be done using CTC. However, some years earlier, following criticism of the use of CTC, a new method using butane was developed (ASTM D5228-92 (2005)) Standard test method for Determination of Butane Working Capacity of Activated Carbon. When an analyst changes to a new standard test method, the question always arises about the relationship of the new result to those obtained with the older method, and so, foreseeing such concern, ASTM has published a correlation graph that enables the old and new results to be viewed on a common scale: CTC activity = 2.55 x butane activity (R^2 = 0.935). This is included in ASTM D5742-95 (2005) Standard Test Method for Determination of Butane Activity of Activated Carbon.